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Technical/Final Report
to
Saskatchewan Agriculture Research Fund
The Development of Techniques and Strategies
of Selection for Grain Yield,
Protein and Kernel Hardness in Wheat
Using Near-Infrared Spectroscopy

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S-85-05-0002/1-85-22

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FINAL REPORT FOR 1987/88 OF THE SECOND YEAR OF
SASKATCHEWAN AGRICULTURE DEVELOPMENT FUND RESEARCH GRANT

(previously referred to as SARF grant #1-85-22)

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The Development of Techniques and Strategies of Selection for Grain Yield,
Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy

Abstract

Our results indicate that near infrared spectroscopy (NIR) can be used in a wheat breeding program to increase efficiency and lower costs required to screen for certain kernel quality characteristics. Kernel hardness and protein are two of the most important quality components of Canadian wheats and laboratory procedures for both are labour intensive and expensive. NIR determinations for kernel protein, hardness and moisture can be done simultaneously and with good accuracy. Complete analysis on a ground sample takes only 30 seconds. However, instrument calibration must be done carefully and sample processing procedures must be strictly followed.

Background and Objectives (unchanged from 1986/87 report)

In order to remain competitive on the world wheat market, Canada must develop wheats which match the quality requirements of the importing countries. For example, high protein wheats tend to yield less grain; therefore, it is poor economics to use such wheats to compete for markets that do not require high levels of protein. Two of the most important quality components of wheat are protein and kernel hardness. However, traditional methods of measuring these components are expensive, labour intensive and slow. Near Infrared (NIR) spectroscopy represents an attractive alternative to the traditional methods as it is rapid and inexpensive. In 1977, R.D. Rosenthal presented a paper at the Amer. Assoc. of Cereal Chemists meetings, discussing the potential of using NIR to measure many agriculturally important constituents (e.g., protein, moisture, starch, etc.). Dr. P.C. Williams has successfully measured protein (1,2), moisture (1), and hardness (2) with NIR. However, NIR still remains more of an art than a routine technique and considerable study is required to ensure that it can be readily adapted by breeding programs. However, the advantages of integrating NIR into Canadian wheat breeding would be considerable.

Great variation exists in the genetic wheat stocks for most quality characteristics. Researchers such as Kushnir and Halloran (Australia) have demonstrated that desirable grain characteristics, such as protein content and kernel weight, can be transferred from ancestral wheats into adapted varieties.

The objective of this research proposal, in general terms, is to increase the efficiency of wheat breeding programs by utilizing NIR technology to measure quality components such as protein and kernel hardness.

Specifically, we plan to:

- (a) Develop and evaluate calibration constants for a near infrared reflect-

ance spectrophotometer suitable for measuring protein content and kernel hardness in wheat.

- (b) Formulate selection strategies to select for grain yield and protein content in both Hard Red Spring and Amber Durum Wheat classes; and grain yield, protein content and kernel hardness in the Canadian Prairie Spring Wheat class (HY320 type).
- (c) Implement evaluation and selection strategies that increase the efficiency of developing improved cultivars of Canada Prairie Spring, Hard Red Spring and Amber Durum Wheat classes.

During the first year of our research it was discovered that the instrument was extremely sensitive to fluctuations in ambient temperature (contrary to claims of the manufacturer). Therefore, a major objective for this period was to determine instrument accuracy and reproducibility after moving it to a constant temperature room.

Experimental Methods:

(a) Plant samples

Canadian Western Amber Durum

Five Triticum diccocus lines which have high protein content but are unadapted to Saskatchewan and low yielding were hybridized previously with DT367, a high yielding line (20% higher than Wascana and Wakooma) but low in protein content. In 1985, the populations from these crosses were grown at Swift Current and Regina and 53 F6 and F8 lines were selected for further testing. During the winter of 1985-86, 265 head rows were grown at Brawley, Calif. to inbreed and multiply seed stocks. In 1986, 127 of the F8 and F10 lines were grown at Swift Current and Regina. In 1987, 63 lines with improved protein content and good agronomic characteristics were grown in replicated tests at up to five locations. Seed samples were collected from each of these lines for each year and each location.

Canadian Prairie Spring

Thirty nine populations were produced previously to achieve the objective of developing high-yielding, sprouting-resistant white seeded cultivars suitable for registering in this new wheat class. In 1985, 1695 hybrid lines were grown at Swift Current and Regina from these 39 populations. From these, 422 lines were selected with 5 head-rows being established for each selected line ($422 \times 5 = 2110$) for multiplication and inbreeding in the Brawley winter nursery. In 1986, 920 lines were selected from the winter nursery and evaluated for performance relative to the CPS goal at Swift Current and Regina. In 1987, 1316 lines were evaluated for performance against the white CPS goal at two locations. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

Canada Western Hard Red Spring

Three populations were produced previously to achieve the objective of developing a hard red spring wheat with elevated protein and/or grain

yield. In 1986, 614 F4 lines were evaluated in replicated tests at two locations. In 1987, 349 F6 lines were progeny tested for elevated yield and/or protein content at two locations. As for the other classes of wheat, grain samples were collected from each of these lines for each location and in each year.

We also received a set of wheat samples, which are used as protein standards, from Dr. P. Williams of the Grain Research Laboratory in Winnipeg. These were used in the calibration of the NIR. We are presently awaiting the arrival of calibration samples for kernel hardness.

(b) Sample processing

An equilibration chamber is used to condition grain samples prior to grinding for NIR analyses. The chamber is maintained at 21 deg C and 50% relative humidity. The samples are stored in envelopes with computer-generated identification labels on them; they are placed in this chamber for at least one week in order to adjust the moisture content of the grain to between 12 and 14%. Grain samples are then ground using a Udy cyclone grinder (Udy Corp., Colorado) fitted with a constant feed adaptor. Only enough samples are removed from the equilibration chamber as can be conveniently ground within a 2 hour period to prevent changes in grain moisture content prior to grinding. Immediately following grinding the samples are placed in metal containers and sealed to prevent moisture changes.

(c) Near infrared

The NIR instrument used is a Neotec 51A (Neotec, Silver Springs, MD). This instrument uses six rotating filters in order to measure absorption at 768 wavelengths. The equation presently being evaluated for protein is the one supplied by Neotec. For kernel hardness, a wavelength of approximately 1620 nanometer is being used (2). The entire spectra of certain sample sets is being stored on a microcomputer.

(d) Lab analyses

Protein is being measured at the Swift Current Research Station using the Kjeldahl procedure (3). Moisture contents are being measured gravimetrically following 24 hours drying at 105 deg C. Kernel hardness is measured at the Winnipeg Research Station; the method involves determination of grinding times (4).

All data are being analyzed with SAS and SASGRAPH (SAS Institute, Cary, NC) running on a Microvax 2000 (Digital Equipment Corp.).

Results and Discussion

An immediate concern following the first year of research was the reproducibility of the measurements. Our results indicated that the reproducibility problems which we had experienced were entirely corrected by moving the instrument to a constant temperature room.

The NIR instrument was supplied with a set of wavelengths and a math treatment

for the prediction of protein in wheat. In order to test the accuracy of this equation we analyzed the set of samples sent to us by the GRL laboratory in Winnipeg. They had determined protein content of each of these samples at least 20 different times using the macro-Kjeldahl procedure (3). The results from the NIR correlated very highly with the true values for this set of samples (linear correlation coefficient > 0.95). Only minor corrections were required to our equation. This were very encouraging results.

The correlation was not as good, however, between NIR predicted protein values and Kjeldahl protein on samples collected from several field trials. Further study indicated that there were two reasons for this.

The first reason was that the laboratory procedure for the determination for protein (i.e., Kjeldahl) was found to be more variable than was previously realized. Through contact with other research establishments we found that the accuracy of the Kjeldahl protein method was of concern to others also. This is very important because the NIR can only be as accurate as the laboratory method used to calibrate it.

The second reason that the correlation was not very good between NIR predicted protein values and Kjeldahl protein on samples collected from several field trials was found to relate to kernel hardness. The equation used by the NIR worked well for hard wheats, however, it overestimated (underestimated) protein of soft wheats by approximately 0.5 percentage unit. The solution was simply to have two equations, one for hard and one for soft wheats.

The results concerning the protein determination are presently very encouraging. The NIR results correlated very highly with laboratory results for wheats from field trials when we (1) ensured that the the laboratory values for protein were highly accurate, and (2) separate equations were used for hard and soft wheats.

We have noticed some discrepancies between different NIR instruments in the results predicted for protein. We believe that this is certainly a possibility which may arise due to the manner in which the instruments are calibrated. For example, it is entirely possible that the protein values from one instrument may be dependent upon kernel hardness, moisture or some unknown characteristic while the values from the other instrument may be independent of these other characteristics. The reason is that NIR instruments from different manufacturers use different regions of the NIR spectrum and different math treatments to predict protein. This means that extreme care must be taken when calibrating an instrument or interpreting results from it.

Results indicate that the NIR can predict kernel hardness very accurately. However, to date we have only calibrated the instrument using grinding-times as the laboratory method. We are presently waiting for a very accurate set of hardness samples to arrive from GRL in Winnipeg.

Conclusions

Kernel protein, hardness and moisture can be accurately determined using the Neotec 51A NIR instrument. However, the following precautions must be taken

for the various constituents:

1. For all analyses the instrument must be maintained in a constant temperature room.
2. For all analyses the laboratory analyses used to calibrate the instrument must be very accurate.
3. For accurate protein and hardness measurements the grain should be equilibrated to constant moisture content prior to grinding. Following grinding, the grain should be stored in air-tight containers.
4. For accurate protein measurements separate equations must be used for hard and soft wheats.

These results indicate that NIR technology should dramatically improve the efficiency of wheat breeding programs concerned with attempting to develop wheats of specific protein and hardness characteristics.

References

1. Williams, P.C., Norris, K.H. and Zarowski, W.S. 1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. *Cereal. Chem.* 59(6):473-477.
2. Williams, P.C. 1979. Screening wheat for protein and hardness by near infrared reflectance spectroscopy. *Cereal Chem.* 56(3):169-172.
3. Amer. Assoc. of Cereal Chemists. Approved methods of the AACC. The Association: St. Paul, Minn. (1962).
4. Baker, R.J. 1977. Inheritance of kernel hardness in spring wheat. *Crop Sci.* 17:960-962.

Implications for the Saskatchewan Agriculture Industry

New applications of NIR technology to the human and livestock feed industries offer opportunities in efficiencies of production. Knowledge and expertise on the use of NIR that has been developed through this project will be available to Saskatchewan agricultural industries that wish to make use of it. Selecting for protein content and/or kernel hardness by use of NIR technology in the CWRS, CWAD, and CPS wheat classes has already started. Wheat cultivars selected for increased grain yield and/or protein content might be available within five or six years.

Brief report in lay language outlining specific practical recommendations for producers or extension personnel

Some NIR instruments used to measure protein content are sensitive to temperature fluctuations, dust and operator. Producers who have protein measured on their grain samples should be cautious about accepting the results provided.

Financial Statement

Reporting for the first one-year contract terminated on July 20, 1987. Therefore, the financial statement for the second year of this contract commences July 21, 1987.

Period July 21, 1987 to Aug. 24, 1988:

Funding from SARF (to present):	\$16,200.00
Final payment (contingent upon final report)	\$ 1,800.00
Total	\$18,000.00

Expenses:

Deficit from previous year	\$ 326.81
Materials and supplies	\$ 1,608.85
Employee costs:	
Salary and employer contributions	\$11,820.40
Workers compensation	\$ 180.05
Service and administrative charges	\$ 840.20
	\$14,776.31

Forecast of expenses to Sept 16/88:

Employee costs:	
Salary and employer contributions	\$ 3,134.80
Service and administrative charges	\$ 90.00
	\$ 3,224.80

Total expenses for period July 21/87-Sept 16/88

\$18001.11

*See continuation of project
under R-88-05-0309*

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FINAL REPORT FOR 1986/87 OF THE FIRST YEAR OF SARF RESEARCH PROJECT
#1-85-22

The Development of Techniques and Strategies of Selection for Grain Yield, Protein and Kernel Hardness in Wheat Using Near-Infrared Spectroscopy

PART I

1. Abstract

Near infrared (NIR) spectrophotometry has potential as an inexpensive and rapid means of measuring protein content and kernel hardness of wheat; this could significantly improve the efficiency of breeding programs. However, at present our knowledge of NIR is very limited. The objective of the present research project is to integrate such an instrument with a wheat breeding program. The results for the first year of this study are promising although most of the emphasis has been on collection of appropriate plant samples and development of methodology. Several plant populations have been developed from parents containing desirable quality attributes. Concerning instrumentation, it was found that to accurately measure kernel hardness using NIR, all samples should be at a similar moisture content prior to grinding; a chamber was therefore modified to equilibrate grain at 12-14 % moisture. A serious problem was discovered concerning the measurement of protein with the Neotec SIA NIR instrument; the instrument was found to be extremely susceptible to fluctuations of the ambient temperature. The NIR was therefore moved to a constant temperature room to overcome this problem. We are presently evaluating the reproducibility of the NIR to measure protein and kernel hardness following the methodology improvements outlined above.

2. Background and Objectives

In order to remain competitive on the world wheat market, Canada must develop wheats which match the quality requirements of the importing countries. For example, high protein wheats tend to yield less grain; therefore, it is poor economics to use such wheats to compete for markets that do not require high levels of protein. Two of the most important quality components of wheat are protein and kernel hardness. However, traditional methods of measuring these components are expensive, labour intensive and slow. Near Infrared (NIR) spectroscopy represents an attractive alternative to the traditional methods as it is rapid and inexpensive. In 1977, R.D. Rosenthal presented a paper at the Amer. Assoc. of Cereal Chemists meetings, discussing the potential of using NIR to measure many agriculturally important constituents (e.g. protein, moisture, starch, etc.). Dr. P.C. Williams has successfully measured protein (1,2), moisture (1), and hardness (2) with NIR. However, NIR still remains more of an art than a routine technique and considerable study is required to ensure that it can be readily adapted by breeding programs. However, the advantages of integrating NIR into Canadian wheat breeding would be considerable.

Great variation exists in the genetic wheat stocks for most quality characteristics. Researchers such as Kushnir and Halloran (Australia) have demonstrated that desirable grain characteristics, such as protein content and kernel weight, can be transferred from ancestral wheats into adapted

varieties.

The objective of this research proposal, in general terms, is to increase the efficiency of wheat breeding programs by utilizing NIR technology to measure quality components such as protein and kernel hardness.

Specifically, we plan to:

- (a) Develop and evaluate calibration constants for a near infrared reflectance spectrophotometer suitable for measuring protein content and kernel hardness in wheat.
- (b) Formulate selection strategies to select for grain yield and protein content in both Hard Red Spring and Amber Durum Wheat classes; and grain yield, protein content and kernel hardness in the Canadian Prairie Spring Wheat class (HY320 type).
- (c) Implement evaluation and selection strategies that increase the efficiency of developing improved cultivars of Canada Prairie Spring, Hard Red Spring and Amber Durum Wheat classes.

3. Experimental Methods:

(a) Plant samples

Canadian Western Amber Durum

Five Triticum dicoccum lines which have high protein content but are unadapted to Saskatchewan and low yielding were hybridized previously with DT367, a high yielding line (20 % higher than Wascana and Wakooma) but low in protein content. In 1985, the populations from these crosses were grown at Swift Current and Regina and 53 F6 and F8 lines were selected for further testing. During the winter of 1985-86, 265 head rows were grown at Brawley, Calif. to inbreed and multiply seed stocks. In 1986, 127 of the F8 and F10 lines were grown at Swift Current and Regina. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

Canada Prairie Spring

Thirty nine populations were produced previously to achieve the objective of developing high-yielding, sprouting-resistant white seeded cultivars suitable for registering in this new wheat class. In 1985, 1695 hybrid lines were grown at Swift Current and Regina from these 39 populations. From these, 422 lines were selected with 5 head-rows being established for each selected line (422 x 5 = 2110) for multiplication and inbreeding in the Brawley winter nursery. In 1986, 920 lines were selected from the winter nursery and evaluated for performance relative to the CPS goal at Swift Current and Regina. Seed samples were collected from each of these lines grown at two locations for two years and also in a winter nursery.

(b) Sample processing

An equilibration chamber is used to condition grain samples prior to grinding for NIR analyses. The chamber is maintained at 21 deg C and 50 % relative humidity. The samples are stored in envelopes with computer-generated identification labels on them; they are placed in this chamber for at least one week in order to adjust the moisture content of the grain to between 12 and 14%. Grain samples are then ground using a Udy cyclone grinder (Udy Corp., Colorado) fitted with

a constant feed adaptor. Only enough samples are removed from the equilibration chamber as can be conveniently ground within a 2 hour period to prevent changes in grain moisture content prior to grinding. Immediately following grinding the samples are placed in metal containers and sealed to prevent moisture changes.

(c) Near infrared

The NIR instrument used is a Neotec 51A (Neotec, Silver Springs, MD). This instrument uses six rotating filters in order to measure absorption at 768 wavelengths. The equation presently being evaluated for protein is the one supplied by Neotec. For kernel hardness, a wavelength of approximately 1620 nanometer is being used (2). The entire spectra of certain sample sets is being stored on a microcomputer.

(d) Lab analyses

Protein is being measured at the Swift Current Research Station using the Kjeldahl procedure (3). Moisture contents are being measured gravimetrically following 24 hours drying at 105 deg C. Kernel hardness is measured at the Winnipeg Research Station; the method involves determination of grinding times (4).

All data is being analyzed with SAS and SASGRAPH (SAS Institute, Cary, NC) running on a VAX 750 minicomputer (Digital Equipment Corp.).

4. Results and Discussion:

When wheats of differing hardness are ground, the resulting grinds have differing particle size distributions. Since the NIR is very sensitive to the particle size of a sample, it is this property which allows the NIR to measure kernel hardness. However, results obtained early in this study indicated that in order to determine kernel hardness accurately by NIR, it was necessary that all grain samples be brought to the same moisture content prior to grinding. The reason for this was that grain moisture content also affects particle size distribution during grinding. Therefore, differing moisture contents at time of grinding could be interpreted as hardness differences. Since most of the NIR results reported in the literature used grain of approximately 12 to 14 % moisture, we decided to bring our grain to this moisture content also, to aid with comparison of results with other groups. According to the literature, the conditions required to adjust unground wheat to 12 to 14 % moisture are 21 deg C and 50% humidity. As we did not have a chamber available which could produce such conditions, one was modified by the addition of a dehumidifier. A study indicated that grain at high (>15 %) or low (<8%) moisture would fall within the 12-14 % range after only a few days in the chamber. Routinely, all samples now remain within the chamber for at least one week prior to grinding.

Several populations, involving several hundred grain samples, were measured for protein content during the fall and winter months of 1986 using the NIR. Although the NIR-protein and Kjeldahl-protein values from some of these tests were highly correlated ($R^2 > 0.9$), results of other tests indicated considerable variation between the lab (Kjeldahl) and NIR protein. A detailed study of the results indicated that we had a problem

with reproducibility from day to day. Considerable time was spent trying to determine the source of this problem. A trip was made to Winnipeg to discuss some of our results with Dr. Phil Williams (Grain Research Laboratory), a recognized expert in the field of NIR. With Dr. Williams cooperation, and discussions with others using similar NIR equipment (at the Brandon Research Station), we established that the instrument was responding to variations of the ambient temperature (even though the specifications for the NIR claim that the instrument is stable to such temperature changes). Recently we were able to move the instrument to a constant temperature room and we are now in the process of evaluating results under these conditions. Dr. Williams is also providing us with monthly grain samples which are analysed for protein content by several labs to help in the calibration of the instrument.

5. Impact

The results from the first year of this study have demonstrated the importance of sample processing and methodology to achieve the accuracy demanded by wheat breeding programs. We have determined that samples should be equilibrated to 12-14 % moisture prior to grinding. We have also identified the need to maintain constant temperature operating conditions for the NIR instrument. These findings have increased the accuracy of measuring the target parameters of kernel hardness and grain protein content. This lays a better foundation upon which to proceed to develop reliable technology to select for these characteristics in a wheat cultivar improvement program

The long term impact of these 1986/87 results will contribute to the development of wheat cultivars better suited to meet the requirements of producers and to increase our competitive edge in international grain markets.

References

1. Williams, P.C., Norris, K.H. and Zarowski, W.S. 1982. Influence of temperature on estimation of protein and moisture in wheat by near-infrared reflectance. *Cereal. Chem.* 59(6):473-477.
2. Williams, P.C. 1979. Screening wheat for protein and hardness by near infrared reflectance spectroscopy. *Cereal Chem.* 56(3):169-172.
3. Amer. Assoc. of Cereal Chemists. Approved methods of th AACC. The Association: St. Paul, Minn. (1962).
4. Baker, R.J. 1977. Inheritance of kernel hardness in spring wheat. *Crop Sci.* 17:960-962.

Financial Statement

As mentioned in a previous Progress report, there was considerable delay involved in signing this first year agreement which resulted in delays of hiring, purchasing and, therefore, results. We did not receive the unsigned contract until June 2, 1986 and then there were further delays with the Swift Current Agricultural and Exhibition Association (to whom we are indebted for the management of financial matters). The result was that we did not receive the first allotment until Sept. 1986. Hopefully, the first allotment for 1987 can be received by July 1987.

Following is the financial statement for the period April 1, 1986 to June 4, 1987:

Funding received from SARF grant	16,200.00
Final payment (contingent upon acceptable final report)	1,800.00
	18,000.00

Expenses:

Materials and supplies	730.60	
Employee costs :		
Salary	9,356.00	
Employer's contribution	3,445.54	
Computer and statistical analyses	600.00	
Travel (trip to Winnipeg)	389.55	
Service charges	395.55	
	14,918.09	14,918.09

Anticipated expenditure to June 30, 1987:

Employee costs:		
Salary	1,291.08	
Employer's contribution	1,100.00 (est.)	
	2,391.08	2,391.08
Total expenses	17,309.17	

Carryover as of June 30/87 (estimate)	690.83
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Part II ABSTRACT

In the world wheat market, as in other markets, exporting countries must match the quality requirements of the importing countries, or sell their product at a discount. For example, it is poor economics to sell a high protein wheat into a market with a quality requirement for low protein wheat. However, presently it is very expensive, labour intensive and time-consuming for wheat breeders to screen all of their wheat lines for protein and kernel hardness. Near infrared instruments have the potential of measuring protein content and kernel hardness at a low cost. However, it involves fairly new and sophisticated technology, and our knowledge is still somewhat limited in this area. The main objective of this study is to attempt to adapt a near infrared instrument to a wheat breeding program in the hope of greatly improving the efficiency and decreasing the expense when breeding to improve the quality characteristics of wheat. Following the first year of this study much of the plant material has been obtained and much has been learned concerning the conditions required for plant processing and instrument operation.